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ORAL CONTRACEPTIVE USE IN WOMEN IS ASSOCIATED WITH DEFEMINIZATION OF OTOACOUSTIC EMISSION PATTERNS

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Abstract—The production of otoacoustic emissions (OAEs) by the cochlea is a sexually dimorphic trait. Although often hypothesized to be influenced by testosterone *in utero*, little attention has been devoted to the possibility that levels of circulating sex steroids in adulthood might modulate the sex difference in OAE production. The purpose of the current study was to investigate whether oral contraceptive (OC) use affects OAE production in women, revisiting a question originally posed by McFadden [(2000) Hearing Research 142:23–33]. Forty-five males and 50 females were tested. The women were retrospectively classified based on whether or not they were using OCs at present. Two types of OAEs were quantified: those produced spontaneously (spontaneous otoacoustic emissions or SOAEs) and those produced in response to click stimuli (click-evoked otoacoustic emissions or CEOAEs). Women currently using OCs showed a defeminized pattern of OAE production: they produced fewer SOAEs, SOAEs with significantly less power, and smaller CEOAE response amplitudes compared with naturally cycling women who were tested irrespective of phase of the menstrual cycle. It is proposed that the observed group difference may be mediated by the interaction of circulating estradiol with estrogen receptor alpha (ERα) or estrogen receptor beta (ERβ) receptors in the cochlea.

Key words: activational, auditory, cochlea, estrogens, testosterone.

Otoacoustic emissions (OAEs) are faint sounds produced by a normally functioning cochlea, which can be detected in the external auditory canal using a highly sensitive microphone (Kemp, 1978). An association between the production of OAEs and normal hearing sensitivity has been found (Probst et al., 1987; McFadden and Mishra, 1993). OAEs are widely considered to be a natural by-product of an amplification mechanism in the cochlea designed to amplify low-intensity sounds, the "cochlear-amplifier system" (Davis, 1983), of which the outer hair cells are an integral component. Several types of OAEs have been identified. Of interest here will be two types: (1) those produced in the absence of external acoustic stimuli (spontaneous OAEs, SOAEs) and (2) those produced in response to the deliberate presentation of acoustic stimuli, either tonal bursts or clicks (click-evoked OAEs, CEOAEs).

A sex difference in OAE production has been found in humans. Females, on average, produce greater numbers of SOAEs, greater overall power of SOAEs, and higher CEOAE response amplitudes compared with males (Bilger et al., 1990; Burns et al., 1992; Penner et al., 1993). This robust sex difference has been observed in neonates, infants, and young children (Strickland et al., 1985; Burns et al., 1992; Morlet et al., 1995), as well as certain adult populations (for review, see McFadden, 2008, 2009), and is most obvious in the first year after birth (Lamprecht-Dinnesen et al., 1998). To explain the sexual dimorphism, it has been hypothesized that exposure to elevated levels of androgens, specifically testosterone, in the male fetus during the critical prenatal window for sexual differentiation masculinizes the auditory system, including the mechanisms responsible for OAE production (i.e., the cochlear-amplifier system), resulting in a diminished capacity to generate OAEs in males relative to females (McFadden, 1993b, 1998, 2002). A right-ear advantage in the production of both SOAEs and CEOAEs also has been reported (e.g., Bilger et al., 1990; Burns et al., 1992; Hall, 2000; McFadden, 2009; Talmadge et al., 1993, but see Collet et al., 1993).

Direct support for the prenatal androgen hypothesis remains limited, owing to the difficulty of studying prenatal effects in humans where the experimental manipulation of testosterone is not ethically permitted, but several lines of indirect evidence do exist. Female dizygotic twins who have male co-twins have been shown to produce male-typical patterns of OAEs, presumably due to exposure to higher-than-normal levels of androgens *in utero* from their male co-twin (McFadden, 1993a). Studies of sexual orientation and OAE production have shown that homosexual females lie intermediate to heterosexual females and heterosexual males with respect to the numbers and powers of SOAEs produced (McFadden and Pasanen, 1999) and CEOAE response amplitudes (McFadden and Pasanen, 1998). The latter finding is congruent with the possibility that homosexual women are exposed to elevated levels of androgens prenatally, resulting in partial masculinization of their brains, and subsequent behavior (see also Hall and Kimura, 1995; McFadden and Champlin, 2000 for partial masculinization of other traits).

A study of spotted hyenas (*Crocuta crocuta*) offers support from a non-human species for a prenatal hormonal effect on OAE production (McFadden et al., 2006). Both female and male hyenas are highly androgenized during prenatal development. Female hyenas not only produce

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Abbreviations: ANOVA, analysis of variance; CEOAE, click-evoked otoacoustic emission; dB, decibels; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; Hz, hertz; kHz, kilohertz; OAE, otoacoustic emission; OC, oral contraceptive; SOAE, spontaneous otoacoustic emission.

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CEOAE response amplitudes similar to those present in male hyenas, but also the prenatal treatment of both female and male hyenas with anti-androgens resulted in stronger CEOAE amplitudes in both sexes. Conversely, prenatal treatment with testosterone propionate has been found to reduce the amplitude of the CEOAE response in female sheep (McFadden et al., 2009). Thus, multiple lines of evidence suggest that prenatal androgens may act to weaken the cochlear amplifiers responsible for OAE production.

Many sexual dimorphisms that are initiated by androgen exposure during the prenatal or perinatal period are subject to further regulation by levels of circulating hormones in adults (Goy and McEwen, 1980). However, with respect to OAEs, research examining the possibility of a superimposed influence of adult steroids has been limited and has yet to firmly establish a role for circulating hormones in OAE production. Recent work by our laboratory confirmed a correlation between CEOAE response amplitudes and circulating levels of testosterone in adult men (Snihur and Hampson, 2012). McFadden et al. (2006) showed that male rhesus monkeys produce CEOAEs with lower response amplitude during the fall breeding season (i.e., elevated levels of sex steroids) compared with the summer non-breeding season (i.e., reproductively quiescent; lower levels of sex steroids). Androgens have been the focus of most existing research because of mounting evidence that they exert organizational effects on the development of the auditory system. However, other hormones might also play a role in the regulation of adult OAEs.

An estrogenic influence has not been demonstrated to date, but would be consistent with several indirect observations. In women, at least two case reports have described an infradian rhythm in the frequencies of emitted SOAEs that approximates the length of the menstrual cycle (changes in OAE numbers or amplitudes were not reported). Three of four women studied by Bell (1992) showed cyclic fluctuation of about 6–14 hertz (Hz) (0.4%) in the frequencies of the SOAEs they emitted and, in a single-case study, fluctuation in one woman's SOAE frequencies was reduced during periods of amenorrhea (Penner, 1995). Monthly fluctuations in SOAEs in females also were observed by Haggerty et al. (1993). Endocrine verification of the menstrual cycle was not provided. McFadden (2000) speculated that oral contraceptive (OC) use also might affect OAE production and tested this hypothesis in a retrospective analysis of SOAE and CEOAE data collected from young women. Modest differences in the means were observed on several OAE measures, but none of these differences were significant. Previously undetected SOAEs were found in a transsexual male while undergoing estrogen replacement (and androgen suppression) before sex-reassignment surgery (McFadden et al., 1998). Furthermore, hearing sensitivity, which shares physiological substrates with OAE production, exhibits variation over the menstrual cycle, with poorer auditory thresholds during menses when ovarian output is lowest (Swanson and Dengerink, 1988). Recent demonstrations of estrogen receptor expression in the mouse, rat, and adult human cochlea (Stenberg et al., 1999, 2001), notably the presence of ligand-dependent estrogen receptor beta (ERβ, a subtype of the estrogen receptor) in the inner and outer hair cells (Meltser et al., 2008), afford a potential mechanism by which circulating estradiol, the dominant estrogen in women of reproductive age, could influence OAE production.

As a step toward defining the role of adult steroid concentrations, the goal of the current study was to investigate whether the use of OCs affects the production of SOAEs and CEOAEs in women as predicted and first tested by McFadden (2000). Although no significant difference between OC users and nonusers was found by McFadden (2000), OC formulations have changed appreciably over time. OCs reliably suppress the ovarian production of estradiol and the rise in progesterone that follows ovulation (Kafrissen and Adashi, 2003). If circulating estradiol levels are, indeed, an important regulator of OAE production, then we predict that the suppression of estradiol through OC use will influence the capacity to generate OAEs in women, as reflected in the number and overall power of SOAEs produced, and the response amplitude of CEOAEs elicited in response to acoustical stimulation.

EXPERIMENTAL PROCEDURES

Participants

Male (n=45) and female (n=50) undergraduates, aged 17–25 years, were recruited to participate in a study of sex differences in the auditory system. Ethnic differences in OAE production have been reported previously (Whitehead et al., 1993); 89% of the present sample was Caucasian, 1% Black, and 10% Asian (including one OC user and three non-OC users). In the present work, the OAE results were the same with and without all ethnic subgroups included, therefore the full data set is reported here.

All volunteers initially underwent standard clinical audiometric screening using a GSI-17 pure-tone air-conduction audiometer, at frequencies from 250 to 8000 Hz, to ensure inner-ear integrity. Individuals who did not pass the screening criterion (i.e., who had audiometric thresholds greater than 25 decibels (dB) hearing level at any of the tested frequencies) were not included.

Eligible participants were classified retrospectively into three groups based on their responses to a demographic and health questionnaire that was given following the OAE testing: males (n=39), females not using OCs at present (female non-OC users; n=26), and females who self-identified as using OCs at present (female OC users; n=20). Females in the OC group used standard low-dose OCs containing 20–30 μg/d of ethinyl estradiol; approximately 90% used OCs containing 25 μg or less (e.g., Alesse 21). About 60% used triphasic OCs that varied in their progestogen content over the 21-day contraceptive cycle but had a fixed dose of ethinyl estradiol (e.g., Tri-Cyclen Lo). The remainder took monophasic formulations. Sexually active females in the non-OC group used other methods of birth control that did not include any form of hormonal contraception (e.g., injections, patch). Because classification into groups took place retrospectively, no attempt was made to assess OAEs at any particular stage of the menstrual cycle. The three groups were well-matched on age: males (M=20.84 y±2.59 SD), female non-OC users (19.65 y±1.83 SD), female OC users (20.09 y±2.25 SD).

The demographics questionnaire also contained items that screened for health conditions previously shown to affect OAE production, such as use of certain prescription drugs and ear or cochlear damage or surgery (McFadden and Plattsmier, 1984;

Probst et al., 1987), which served as exclusionary criteria. Retrospective assignment to groups ensured that the experimenter was blind to women's OC status throughout the OAE recording, identification, and scoring procedures.

General procedure and equipment

All testing took place in a darkened, quiet testing room between 1400 h. and 2000 h. OAEs were measured in both ears, independently. The ear tested first and the type of OAE tested first (SOAE or CEOAE) were separately counterbalanced.

Participants reclined in a sofa chair during OAE recording. The external auditory canal was inspected for any debris or blockage using an otoscope (Welch Allyn MacroView 23820, Mississauga, Ontario, Canada). A foam ear-tip attached to an Etymotic ER-10B low-noise microphone system then was fitted into the external auditory canal. The microphone system consisted of two small-diameter coupling tubes protruding ~2 mm into the auditory canal, connected to an Etymotic ER-2 miniature insert earphone. This microphone system served two functions: (1) to act as a conduit for the delivery of acoustic stimuli to the inner ear during the CEOAE recordings and (2) to allow the detection of emissions during the SOAE and CEOAE testing. For better measurements, participants remained still during a 15-min habituation period before commencing the SOAE and CEOAE testing (e.g., Zurek, 1981; McFadden, 2000; Whitehead, 1991).

Emissions detected by the low-noise microphone system during the SOAE and CEOAE testing were amplified and filtered, then stored digitally on a laptop computer for off-line analysis and identification. An ER 10-72 pre-amplifier received output from the microphone system and passed it along to a custom-built amplifier/filter. Output responses were amplified by 30 dB and high-passed above 400 Hz to eliminate any extraneous bodily or environmental noises present during the recording. The output was digitized using an analog-to-digital converter (National Instruments, DAQ AI-I6XE-50), then stored on a Macintosh G4 Powerbook (OS 9.2). Data collection and off-line analysis of the OAE data were accomplished using custom-written software in LabVIEW (National Instruments, Austin, TX, USA). Programs and methodology were obtained courtesy of E.G. Pasanen from the University of Texas at Austin.

SOAE detection and identification

Participants were instructed to remain as still as possible during each recording interval. For SOAE measurement, four 30-s recordings of spontaneous activity were taken from each ear. These raw SOAE recordings were then digitized with 16-bit resolution at a sampling rate of 25 kilohertz (kHz) and stored on the computer hard drive. All further transformations, detection, and analyses of SOAEs were conducted off-line. The quietest 150 time segments (655 ms in length; approximately 75% overlap with adjacent time segments) from the entire 2-min recording from each participant were selected, and fast Fourier transforms of these segments were computed and averaged in the frequency domain. This averaged spectrum corresponded to approximately 25% of the original 2-min sample. SOAE peaks were identified using an automated computer algorithm (for details see Pasanen and McFadden, 2000). To be defined as an SOAE, all the following criteria had to be met: (1) the frequency of the peak resided between 1000 Hz and 9000 Hz; (2) the peak was at least five standard deviations above the averaged spectral baseline; and (3) the peak was not within 0.1 octaves of a stronger SOAE, as it has been suggested that true SOAEs cannot exist closer than 0.1 octaves of one another (Zwicker, 1990). Once identified as an SOAE, the peak was converted to sound-pressure level (SPL) and stored for statistical analysis. The dependent variables computed were the number of SOAEs produced, overall SOAE power summed across all the SOAEs identified in each ear (or across both ears), and the power per SOAE.

CEOAE detection

CEOAE detection was performed for two click intensities (75 peSPL and 69 peSPL) in each ear. These click levels corresponded to the peak amplitude of a 1000-Hz tone at the specified intensities and were generated as rarefaction DC pulses (97.7 μs in duration) by the laptop sound output system at a sampling rate of 44.1 kHz. Each click intensity was calibrated before each CEOAE recording procedure. Similarly, the level of ambient noise present in the ear being tested was sampled and averaged to establish individual noise thresholds. Following click calibration and noise-floor threshold determination, clicks were presented at a nominal rate of 10 per second through the microphone system. Evoked responses to the presentation of the clicks were recorded unless the ambient noise during click presentation exceeded the pre-determined noise threshold by 0.25 standard deviations or more; if this occurred, presentation of subsequent clicks was delayed until the ambient noise returned to an acceptable level. Cochlear output was digitally sampled at 48 kHz, synchronized to the click stimulus as recorded directly from the sound output of the computer, and band-pass filtered at 1–5 kHz. To avoid interference from any acoustical ringing that resulted from click presentation, a 6-ms delay was applied to the beginning of analysis of each response. This corresponded to a 2-ms delay in the physical recording after presentation of the click, and a 4-ms delay during the off-line analysis. The click-evoked response used for statistical analysis consisted of an averaged response from 250 of the quietest click responses (20.48 ms in duration) with the 4-ms delay applied. The click-evoked response was then converted from the root-mean square amplitude into decibels sound-pressure level (dB SPL), and the latter was the dependent variable used for all subsequent statistical analyses involving CEOAEs (for procedure, see McFadden and Pasanen, 1998).

Saliva collection and hormonal quantification

The primary mechanism of OC action is to inhibit pituitary gonadotropins (Kafrissen and Adashi, 2003), and thus, endogenous production of estradiol by the ovaries. Estradiol concentrations are suppressed to levels typical of menses or below (Gaspard et al., 1983). Bioavailable estradiol, the fraction of the hormone not bound to sex hormone-binding globulin (SHBG), is even lower and challenges the technical limits of detection by conventional assays in serum or saliva. Most OCs also reduce bioavailable testosterone levels (Wiegratz et al., 2003), but the effects are more variable. Therefore, we quantified bioavailable testosterone using saliva to evaluate whether any changes in OAEs that result from OC use could be explained by testosterone (instead of the suppression of estradiol levels).

Saliva was collected immediately before the SOAE and CEOAE recordings. Participants refrained from eating, drinking (except water), smoking, or brushing their teeth for 1 h before collection. Before providing saliva, the mouth was rinsed with water. A sugarless gum (Trident™ peppermint), known to be inert in the assay employed here, (cf. van Anders, 2010), was used to stimulate saliva flow. The saliva was collected into a polystyrene culture tube pre-treated with sodium azide to prevent bacterial degradation. The samples were kept at −20 °C until assay.

Each sample was analyzed by radioimmunoassay, in duplicate, following a double ether extraction. A ^{125}I Coat-a-Count kit for total testosterone (Diagnostic Products Corporation, Los Angeles, CA, USA) was modified for saliva according to an established protocol (Moffat and Hampson, 1996; Puts et al., 2010). Of 10 immunoassay kits available commercially, the Diagnostic Products kit yielded the best correlation with a gas chromatography/

mass spectrometry measure of testosterone in women (Taieb et al., 2003). The lower limit of detection for the assay was 2.5 pg/ml. The average intra-assay coefficient of variation was 5.2%.

Statistical analysis

Mixed-effects analysis of variance (ANOVA) with ear and, where applicable, click level as repeated measures were used to separately analyze group differences in SOAE production and CEOAE response amplitude. One-way ANOVA was used to analyze group differences in SOAE power and testosterone concentrations. Fisher's Least Significant Difference test was used to perform post hoc pairwise comparisons. Effect sizes were expressed using Cohen's *d* (Cohen, 1977). By convention, an effect size of $d=.50$ is considered a medium effect and .80 or above is considered large (Cohen, 1977).

RESULTS

SOAEs

The hypothesis regarding an effect of OC use on SOAE production in females was supported. Female OC users exhibited significantly fewer SOAEs compared with female non-OC users.

With respect to the total number of SOAEs produced, a significant main effect of group was found [$F(2,82)=7.47$, $P=0.001$; see Fig. 1], with female non-OC users producing a greater number of SOAEs summed across both ears compared with female OC users ($P=0.005$) and males ($P<0.001$). Effect sizes for the differences between the non-OC females and OC females and between non-OC females and males were $d=0.95$ and 0.93, respectively. The difference between non-OC females and males confirms the sex difference in SOAE production that has been reported previously (e.g., McFadden and Pasanen, 1999). No significant difference was found between the female OC users and males. A right-ear advantage in SOAE production was evident, with a greater number of SOAEs produced in the right ear than the left ear [$F(1,82)=11.34$, $P=0.001$; see Fig. 2]. The ear advantage was seen most clearly among the female non-OC users, though the interaction between group and ear was only marginally significant [$F(2,82)=2.53$, $P=0.086$]. The group differences observed in the current study are in the same direction as the means found in McFadden (2000); however, the magnitude of the difference between non-OC and OC users in the McFadden study ($d=0.31$) was smaller than that found in the current study.

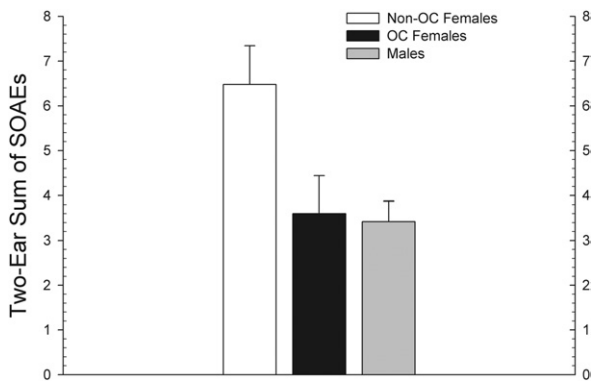

Fig. 1. Total number of SOAEs produced in both ears. Female non-OC users ($n=26$) produced significantly greater numbers of SOAEs than either female OC users ($n=20$) or males ($n=39$). Error bars represent standard error of the mean (SEM).

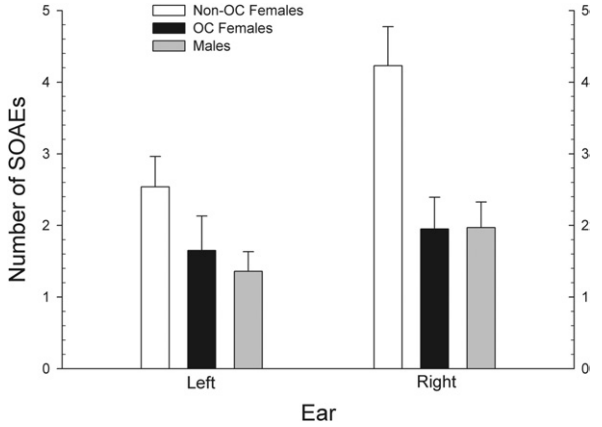

Fig. 2. Number of SOAEs produced in the right and left ears. An overall right-ear advantage was observed, most prominently among female non-OC users. Error bars represent SEM.

Participants who did not produce any SOAEs were not included in the analyses of SOAE power. A significant difference in overall SOAE power, summed across both ears, was found among the three groups, $F(2,69)=8.62$, $P<0.001$ (Fig. 3). Post hoc comparisons revealed that female non-OC users produced SOAEs with greater power than males ($P<0.001$) or female OC users ($P=0.03$), whereas the mean for OC users was shifted in the male direction and was not significantly different from the male group. Effect sizes for the group differences between non-OC females and OC females and between non-OC females and males were $d=0.83$ and $d=1.11$, respectively. This pattern was mainly attributable to power in the right ear, $F(2,61)=4.87$, $P=0.011$. Female non-OC users showed greater overall power in the right ear than males ($P=0.004$) or female OC users ($P=0.033$). There was no significant difference between OC users and males

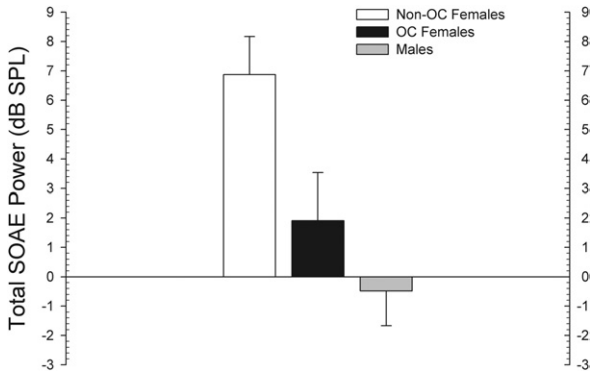

Fig. 3. Total power of all SOAEs produced in both ears. Female non-OC users ($n=23$) produced SOAEs with greater power than female OC users ($n=14$) and males ($n=34$). Error bars represent SEM. Because the y-ordinate represents a logarithmic scale, the 0 value does not represent absolute 0.

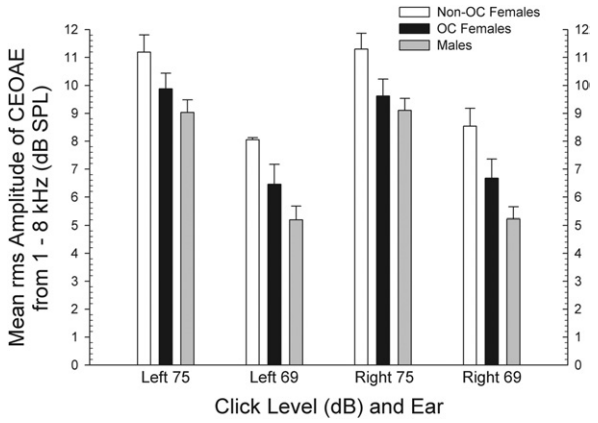

Fig. 4. Mean CEOAE response amplitude in the left and right ears at two click levels (75 and 69 dB SPL). At 69 dB, the female non-OC users (n=24) showed significantly greater response amplitudes than either female OC users (n=18) or males (n=37). At 75 dB, the difference between non-OC users and OC users was marginally significant. Error bars represent SEM.

(P=0.746). Group differences in the left ear were not significant, $F(2,50)=0.94$, $P=0.397$.

To disambiguate whether the difference in overall power more likely resulted from larger numbers of SOAEs or larger amplitudes of the individual OAEs produced, ANOVA was performed using the power per SOAE as the dependent variable. Although the rank ordering of the group means was the same, power per SOAE considered on its own did not significantly differentiate the three groups, $F(2,60)=0.79$, $P=0.458$. Because the group difference in overall SOAE power was not independent of SOAE number, this variable was not considered further.

CEOAEs

It was hypothesized that females using OCs would differ in the amplitude of their click-evoked responses compared with females not currently using OCs (*cf.* McFadden, 2000). Fig. 4 shows the average CEOAE response amplitude in female non-OC users, female OC users, and males for all ear and click level combinations. A significant main effect of group [$F(2,75)=8.89$, $P<0.001$] and of click level [$F(1,75)=621.73$, $P<0.001$] was found. Female non-OC users produced the greatest CEOAE response amplitudes, whereas males produced the lowest amplitudes ($P<0.001$ by post hoc test). There was no significant difference between the ears. Significant interactions also were found between click level and group [$F(2,75)=5.97$, $P=0.004$] and between click level and ear [$F(1,75)=5.30$, $P=0.024$]. Tests of simple main effects were used to break down the interaction between click level and group. At 69 dB, the non-OC females showed significantly greater response amplitudes than either OC females ($P=0.035$) or males ($P<0.001$), with effect sizes of $d=0.54$ and $d=1.13$, respectively, whereas at 75 dB the difference between non-OC females and OC females was marginally significant ($P=0.076$, $d=0.55$). In comparison, the effect size for the difference between non-OC users and OC users in McFadden (2000) was $d=0.10$. In the present data, OC females did not differ significantly from males at either intensity ($P=0.099$ and $P=0.194$ for the two click levels, respectively).

Although SOAEs and CEOAEs are both generated by the cochlear amplifier system, the underlying mechanisms, although overlapping, do include unique sources of variance (McFadden et al., 1996; cf. McFadden, 2000). Effect sizes thus can differ for CEOAEs versus SOAEs, but in the present study, both types of OAE showed a significant contraceptive effect.

Testosterone

Salivary testosterone concentration differed significantly among the groups, $F(2,90)=227.56$, $P<0.001$. As expected, males ($M=83.52$ pg/ml$\pm$22.14 SD) had significantly higher testosterone levels than females (both Ps<0.001). All the OC users had testosterone concentrations that fell within the detectable range of the assay. A post hoc t-test confirmed that OC users had significantly lower testosterone ($M=10.79$ pg/ml$\pm$3.13 SD) than the female non-OC users ($M=17.02$ pg/ml$\pm$4.06 SD), $t(46)=34.50$, $P<0.001$, consistent with the known suppressive effects of OCs on testosterone production (Bancroft et al., 1991).

Testosterone was not significantly correlated with any of the OAE variables, in either of the two female groups.

DISCUSSION

In the present study, OC users were found to produce significantly smaller numbers of SOAEs, SOAEs with less total power and less power in the right ear particularly (although this finding was not statistically independent of the difference in SOAE number), and had weaker CEOAE response amplitudes compared with female non-OC users. For each of the OAE variables, OC users showed a pattern that was shifted in a direction away from the pattern typically seen in non-OC females. That is, they were muted or diminished in their OAE output and thus, may be considered defeminized with respect to this particular trait. The term “defeminization” is used by neuroendocrinologists to denote the reduction in a female-typical characteristic (Breedlove and Hampson, 2002).

Female OC users in the present study did not demonstrate significant sex differences and resembled males in each measured element of OAE production. In contrast, the expected sexual dimorphism in both SOAEs and CEOAEs was confirmed when female non-OC users were compared with males (Strickland et al., 1985; Burns et al., 1992; McFadden, 2008, 2009; Morlet et al., 1995; Penner et al., 1993). The sex difference and, by implication, the hormonal effect is often more pronounced in the right ear than the left (Bilger et al., 1990; Burns et al., 1992; Hall, 2000; McFadden, 2008, 2009; Talmadge et al., 1993), as mirrored in the ear differences found in the present study. Though an organizational effect of prenatal androgens on the cochlea might exist, and can explain the existence of sex differences in OAE production in prepubertal children, the fact that the sex difference was attenuated so markedly

among women choosing to use OCs is consistent with the hypothesis that the adult hormonal milieu exerts an important influence on patterns of OAE production.

Despite retrospectively combining data from two earlier published studies, McFadden (2000) was unable to confirm a statistically significant difference between OC users and nonusers on four different measures of OAE strength. The direction of the group means, however, matched the present results. The difference between the current results and the smaller effect sizes seen by McFadden (2000) may be attributable to changes in the formulations of OCs that have occurred over the intervening 20 years, in that the OCs currently available are exceedingly low in estrogen activity, as indicated by reports of decreased bone density in young women who have been using OCs for an extended period compared with nonusers (Teegarden et al., 2005). Although older as well as recent OC formulations effectively suppress endogenous estradiol production by the ovaries, older contraceptive formulations contained a higher estrogen content (35–50 μg/d compared with 20–25 μg/d now), and thus, supplied more exogenous estrogen in the form of ethinyl estradiol. Ethinyl estradiol, too, is active at the estrogen receptor. If our hypothesis is correct that circulating estrogen normally facilitates the production of OAEs by the female cochlea, then greater damping of the cochlear mechanisms responsible for SOAE and CEOAE production is to be expected under current OC formulations because of the greater reduction in estrogen activity associated with current than older OCs.

The primary mechanism of contraceptive action is the suppression of circulating estradiol and, secondarily, progesterone by OCs. But testosterone levels also are substantially decreased in OC users. Thus, one question that arises is whether decreased testosterone can explain the observed effects of OCs on OAE production. This is unlikely. OAEs were not correlated with testosterone levels in the present data, either in the OC or non-OC females and, in studies of the prenatal effects of testosterone, including animal studies where testosterone levels were manipulated experimentally (e.g., McFadden et al., 2009), as well as studies of adult testosterone (McFadden et al., 2006; Snihur and Hampson, 2012). The direction of testosterone's effect is to inhibit the production of OAEs (i.e., higher testosterone is associated with diminished OAE production). In the current study, OC users showed the reverse pattern whereby testosterone was decreased by OC use but so were OAEs. It is improbable that testosterone would be associated with diminished OAE production in numerous prior studies yet exert the opposite effect here.

A plausible explanation for the observed results is that estradiol, the primary form of estrogen that is produced in copious quantities by the ovaries in naturally cycling females, is actively involved in regulating OAE production in the female cochlea. Ovarian production of estradiol is inhibited by the use of OCs. Thus, we propose that ovarian-derived estrogen in the circulation of non-OC women acts to facilitate the cochlear amplifiers—the active mechanism along the basilar membrane that magnifies membrane displacement at low sound-pressure levels—whereas women using OCs lack this hormonally mediated facilitation. The result is weakened OAE production in OC users.

Recent studies support a potential role for estradiol in normal cochlear functioning. Meltser et al. (2008), for example, showed that ERβ (an estrogen receptor subtype) in the mouse cochlea is involved in auditory sensitivity and protection from acoustic trauma, suggesting that estradiol may exert prophylactic effects on hearing. Estradiol protects against age-related hearing loss (Simonoska et al., 2009), and aging women receiving hormone replacement therapy tend to have better hearing than women not on therapy (Hultcrantz et al., 2006). ERα and ERβ expression has been described in the mouse, rat, and adult human cochlea, including the outer hair cells, raising the probability that estradiol actively affects cochlear function (Stenberg et al., 1999, 2001). Because previous work has established a relationship between hearing sensitivity and OAE production (Probst et al., 1987; McFadden and Mishra, 1993), it is conceivable that the group differences in OAEs observed among women in the present study are due to a difference in estradiol availability to bind to ligand-dependent receptors in the inner ear.

A few previous studies of humans and other primates have implicated estrogen indirectly in OAE production. The possibility that estrogen modulates cochlear function has not received dedicated research attention, however, and as a result, most existing evidence is circumstantial. The acoustic frequencies of emitted SOAEs have been reported to fluctuate with the menstrual cycle (Bell, 1992; Haggerty et al., 1993; Penner, 1995), although studies are limited to case reports, often without adequate endocrine validation. SOAE frequencies were the highest near the suspected time of ovulation, with some evidence of a second maximum between ovulation and menses (Penner, 1995), a pattern that would support an estradiol-driven effect. The prospect of an estrogen-dependent mechanism is further supported by a case study of a transsexual male undergoing estrogen replacement therapy before sex-reassignment surgery, in whom SOAEs appeared at frequencies where there previously were none (McFadden et al., 1998). Although not conclusive, these studies are consistent with the possibility that elevated levels of estradiol are associated with enhanced OAE production, whereas lower levels are associated with diminished OAEs. An effect of estradiol on OAE production would complement studies showing effects of estrogen on the auditory brain stem response (ABR) (Coleman et al., 1994; Serra et al., 2003; Khaliq et al., 2005). Importantly, and consistent with the present findings, McFadden (2000) found several measures of ABR amplitude and latency were significantly defeminized among women using OCs, relative to female nonusers.

The mechanism by which estradiol might affect OAE production is unknown. However, the motility of the outer hair cells, considered to be important to OAE production, is controlled by acetylcholine (Frolenkov, 2006), a transmitter known to be modulated by estradiol levels (for review see Gibbs, 2010). The outer hair cells comprise the effector arm of the cochlear amplifier system. Acetylcholine stimulation hyperpolarizes the cells and produces increased small-signal gain and increased magnitude of the motile response (Dallos et al., 1997), especially in more basal (as

opposed to apical) hair cells. Because estradiol can increase cholinergic function, increased hearing sensitivity and an increased probability of production of OAEs might be expected to occur under higher estrogen conditions.

Although differences between OC users and nonusers in the present study were medium to large in terms of the effect size, it should be emphasized that we did not attempt to control the phase of the menstrual cycle during which the naturally cycling women were tested. This was not feasible because testing in the present study was done blind, and classification into OC users and nonusers occurred only retrospectively. Estrogen levels vary greatly over the normal cycle, therefore, the present group difference might be an underestimate of the true size of the maximum OC user/nonuser effect on OAEs. A logical next step would be to evaluate OAE production at high- versus low-estrogen phases of the menstrual cycle.

We have assumed that if ovarian hormones play a role in influencing OAE production, they do so by direct interaction with the outer hair cells in the cochlea via a receptor mechanism. However, it is possible that an indirect effect of the hormonal changes induced by OCs could be responsible for changes in OAE production. In normally cycling women, basal metabolic rate increases after ovulation, reflecting the thermogenic effects of progesterone. This will be absent in OC users, where ovulation is suppressed (Kattapong et al., 1995). Hall (1992) has described an influence of body temperature on certain auditory properties, including auditory brain stem responses, but clinical studies have shown no changes in OAEs except under extreme departures from normothermia. Therefore, it is unlikely that body temperature differences owing to OC use could influence SOAEs and CEOAEs (for further discussion, see McFadden, 2000), although other secondary mechanisms with effects on OAEs may yet be identified.

The current study offers novel support for an effect of adult reproductive steroid levels on OAE production. Women using OCs were defeminized, relative to nonusers, with respect to each of the OAE variables we measured. These findings are theoretically significant because effects of circulating levels of hormones on the cochlea are largely unexplored, and, at a broader level, the possibility that sex steroids may influence auditory function is not widely appreciated. These findings open a door toward future studies of adult hormones in the auditory system. Our results may have applied implications for audiologists who use OAEs clinically to assess inner ear integrity, and they draw attention to an unanticipated consequence of OC usage. Because OAEs share physiological substrates with hearing sensitivity, mild diminution in acuity during OC use would not be surprising. Confirmation of this possibility should be one focus of future research endeavors, along with the use of a repeated-measures experimental design to confirm the reversibility of the ear effects after OC use is discontinued.

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